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Activity of aminocandin (IP960) compared with amphotericin B and fluconazole in a neutropenic murine model of disseminated infection caused by a fluconazole-resistant strain of Candida tropicalis

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Objectives: To compare the activity of aminocandin (IP960), a new echinocandin with broad-spectrum *in vitro* activity against *Aspergillus* and *Candida* spp., with that of amphotericin B and fluconazole in a temporarily immunocompromised murine model of disseminated candidiasis.

Methods: Mice were rendered neutropenic with cyclophosphamide and infected intravenously 3 days later with a fluconazole-resistant *Candida tropicalis* strain. Mice were treated with intraperitoneal amphotericin B (5 mg/kg/dose), oral fluconazole (50 mg/kg/dose), intravenous aminocandin (0.1–5 mg/kg/dose) or solvent control for 9 days. Mice were observed for survival and survivors were sacrificed 11 days post-infection. Kidneys, liver, brain and lungs were removed for semi-quantitative culture.

Results: Control mice had 90–100% mortality. After infection with *C. tropicalis*, aminocandin 2.5 and 5 mg/kg/day and amphotericin B yielded 80% survival; aminocandin 1 mg/kg/day yielded 70% survival; aminocandin 0.25 and 0.1 mg/kg/day yielded 30% and 20% survival, respectively; and fluconazole 50 mg/kg/day and control regimens yielded 10% and 0–10% survival, respectively. Aminocandin 2.5 and 5.0 mg/kg/day and amphotericin B were superior in reducing mortality compared with aminocandin 0.25 and 0.1 mg/kg/day, fluconazole and controls ($P < 0.047$). The only regimen to reduce organ burdens below detectable levels was amphotericin B, which cleared 40% of mice. All organ burdens in the aminocandin 1.0, 2.5 and 5.0 mg/kg/day and amphotericin B regimens were significantly lower than other groups ($P < 0.02$).

Conclusions: The data demonstrate that aminocandin at doses of ≥ 1.0 mg/kg/day is as effective as amphotericin B at improving survival and reducing organ burdens in this murine model of disseminated *C. tropicalis*.

Keywords: antifungals, mice, echinocandins, *C. tropicalis*

Introduction

Aminocandin (IP960 previously known as HMR3270) (Figure 1) (Indevus, Lexington, Massachusetts, USA) is a new echinocandin that demonstrates broad-spectrum antifungal activity against both *Candida* spp. (including species resistant to azoles and amphotericin B) and *Aspergillus* spp. (including strains resistant to itraconazole).[1-4] Like other members of the class, aminocandin is a lipopeptide that is not metabolized by the liver and unlike the azoles is not a substrate, inhibitor or inducer of the cytochrome P450 enzymes.[5,6]

Pharmacodynamic studies of aminocandin demonstrated concentration-dependent killing with peak/MIC ratios of at least 4 required to be protective in disseminated *Candida albicans* murine infections.[1]

In this study, we compared the activity of aminocandin with that of amphotericin and fluconazole in a temporarily immunocompromised murine model of disseminated candidiasis caused by a fluconazole-resistant strain of *Candida tropicalis*.

Materials and methods

Test strain

C. tropicalis FA1572 was isolated from a throat swab of a clinical sample.[7] The strain was maintained on a slope of Oxoid Sabouraud

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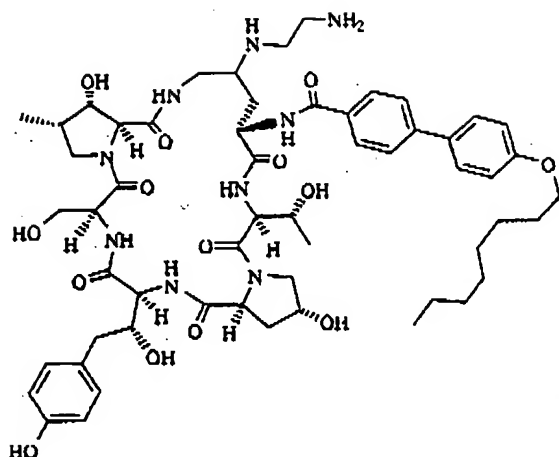
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Figure 1. Chemical structure of aminocandin

dextrose agar (Oxoid Limited, Basingstoke, UK) supplemented with 0 05 g/L chloramphenicol. Long-term storage was at −70°C in nutrient broth (Oxoid) supplemented with 15% glycerol (Sigma-Aldrich, Poole, Dorset, UK).

The *in vitro* sensitivity of the isolate was tested on three occasions according to the AFST-EUCAST guidelines and read at 24 h.[8-10] Minimum fungicidal concentrations (MFCs) were determined by culturing 100 µL from each well in the microdilution plate that had no visible growth; the MFC was taken as the first well with less than 5 cfu.

Animals

All mice included in this study were part of ongoing studies performed under UK Home Office project licence PPL40/1523 entitled Invasive Fungal Infections. Male CD1 mice, 4–5 weeks old and weighing between 18–20 g were purchased from Charles River UK Ltd (Margate, Kent, UK). The mice were virus-free and were allowed free access to food and water. Mice were randomized into experimental groups of 10 per treatment. Each cage was inspected at least four times daily.

Immunosuppression

Cyclophosphamide (Sigma) was administered intravenously (iv) via the lateral tail vein to all animals at a dose of 200 mg/kg. A state of profound neutropenia was achieved 3 days after administration of the drug. White cell counts began to recover 4 days after this nadir.[14]

Preparation of inoculum

The isolate was thawed then incubated for 2 days on Sabouraud dextrose agar One colony was transferred into 25 mL of Sabouraud dextrose broth and incubated on an orbital mixer for 18 h at 37°C, washed twice in saline, then finally resuspended in saline and its density adjusted by spectrophotometry at 490 nm.

Infection of mice

Prior to this experiment, an inoculum-finding study (LD$_{90}$) for this isolate was performed using iv injections of a range of inocula. The LD$_{90}$ inoculum was 2.25 × 10^4/g of mouse weight (i e a 20 g mouse required an inoculum of 4 5 × 10^5 blastoconidia). Mice were infected with the LD$_{90}$ dose on day 0 via the lateral tail vein (3 days post-immunosuppression). Post-infection viability counts were performed

to ensure the correct inoculum had been given. All experiments were performed once.

Antifungal therapy

Deoxycholate amphotericin B (Fungizone, E.R. Squibb, Hounslow, Middlesex, UK) was dissolved in 5% glucose (Baxter Healthcare, Norfolk, UK) to a stock concentration of 5.0 mg/mL. The stock solution was stored at 4°C for up to 7 days before use and was further diluted with 5% glucose for use. Amphotericin B (5 mg/kg) was administered intraperitoneally (ip) on days 1, 2, 4 and 7.[12]

Fluconazole (Pfizer Ltd, Sandwich, Kent, UK) was dissolved in sterile saline plus 0.03% Noble agar (Oxoid) to provide a 50 mg/kg dose. Fluconazole was prepared daily immediately before use and administered by gavage once daily for 9 days.

Aminocandin powder (Aventis, Romainville, France) (13.88 mg; equivalent to 12 5 mg of active drug) was dissolved in 1 mL of sterile water The stock was further diluted in 5% glucose as required and was stored for up to 48 h at 4°C before use. Aminocandin (5, 2.5, 1, 0.25 and 0.1 mg/kg) was administered as a bolus iv once daily for 9 days.

Control mice were infected and received 5% glucose iv or saline plus 0.03% agar by gavage with no active treatment for 9 days

Experimental endpoints

Mice were examined four times daily. Any infected animals with severely reduced mobility, unable to reach the drinker or otherwise in substantial distress were humanely terminated. On day 11 of the experiment all surviving mice were humanely terminated.

Organ culture

The kidneys, liver, brain and lungs were removed aseptically and transferred into 2 mL of sterile phosphate buffered saline (BDH, Poole, Dorset, UK). The organs were homogenized in a tissue grinder (Polytron, Kinematica AG, Luzern, Switzerland) and colony counts determined using serial 10-fold dilutions plated on the surface of the plates. The plates were incubated at 37°C and examined daily for 5 days. This method detected *C. tropicalis* at >30 cfu/organ.

Statistical analysis

Mortality data were analysed using the Log-rank (Peto) test in which *P* values reflect the χ^2 for equivalence of death rates.

Culture data were analysed using the Kruskal–Wallis pairwise comparisons test (Conover–Inman).[13] All data analysis was performed using the computer package StatsDirect (Ashwell, Herts UK)

Results

In vitro susceptibility

The MIC and MFC values for FA1572 were: amphotericin B, 0.0039 and 0.06 mg/L, fluconazole, >128 and >128 mg/L; aminocandin, 0.06 and >0.5 mg/L, respectively.

In vivo results

The mortality curves shown in Figure 2 demonstrate that FA1572 caused lethal infections in mice. Mice receiving no active treatment had either 90% or 100% mortality (0 03% agar and 5% glucose, respectively). No treatment group had 100% survival. Aminocandin at ≥2.5 mg/kg/day and amphotericin B were superior in terms of survival to aminocandin ≤0.25 mg/kg/day, fluconazole and

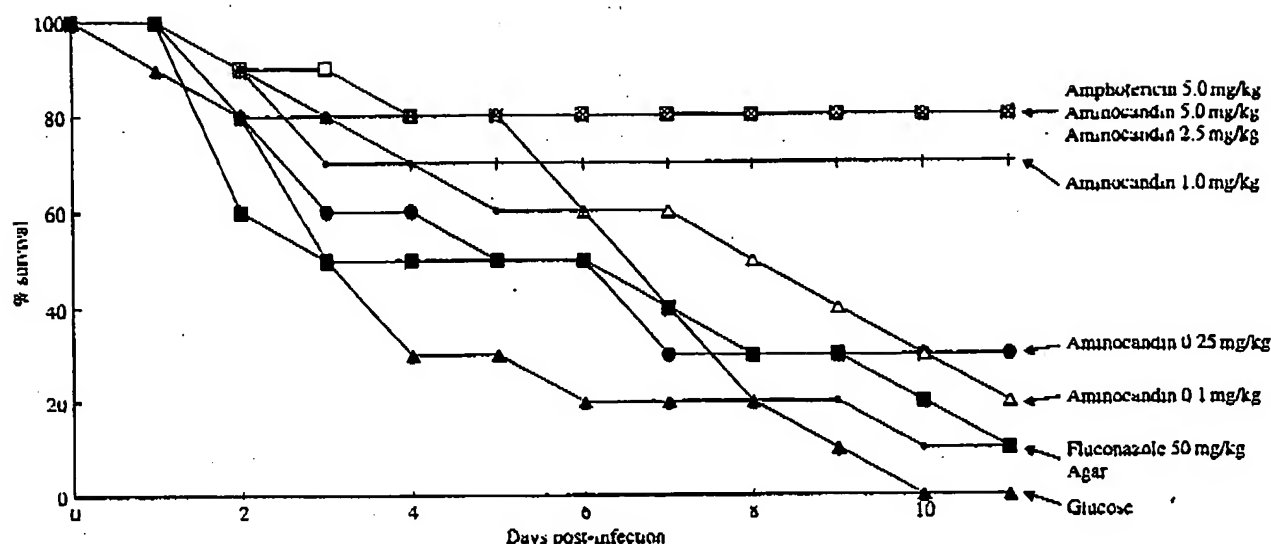## *In vivo* activity of aminocandin in a murine model

Figure 2. Plot of cumulative mortality against time in a murine model against *Candida tropicalis* FA1572. Open squares, aminocandin 5 mg/kg iv; open diamonds, aminocandin 2.5 mg/kg iv; plus symbols. aminocandin 1.0 mg/kg iv; large filled circles. aminocandin 0.25 mg/kg iv, open triangles, aminocandin 0.10 mg/kg iv; asterisks, amphotericin B 5 mg/kg ip. filled squares, fluconazole 50 mg/kg oral; small filled circles, agar oral; filled triangles, glucose iv

controls (*P* ≤ 0.05). Aminocandin 1.0 mg/kg/day was superior to fluconazole and controls (*P* ≤ 0.026).

All the mice that were killed before day 11 had high counts in all organs, demonstrating widespread overwhelming infection. The only treatment to clear organ burdens totally was amphotericin, which cleared 40% of mice. Aminocandin at ≥1.0 mg/kg/day and amphotericin B were superior in terms of organ burden (all organs) to aminocandin ≤0.25 mg/kg/day, fluconazole and controls (*P* ≤ 0.02).

Discussion

Until recently, the only options for treating invasive fungal diseases were associated with dose-limiting toxicity, poor absorption or gaps in their antifungal spectrum. Many of these weaknesses have been addressed by the introduction of new triazoles and echinocandins, but even with these new agents efficacy is still far from ideal with attributable mortality rates still in excess of 30%. There is therefore still a need for further highly effective antifungal agents.

Aminocandin (IP960) is a semi-synthetic fermentation product from *Aspergillus sydowi*.[6] Its chemical structure 1-[4-[(2-aminoethyl)amino]-N2-[(4'-octyloxy)[1,1'-biohenyl]-4-yl]carbonyl]-L-ornithine]-4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine-echinocandin B dihydrochloride (Figure 1) has some similarities with the other members of the echinocandin class It has protein binding of >99%,[1] which differs slightly from caspofungin (97%) and anidulafungin (85%). but probably not micafungin (99.9%).[6] Activity has been demonstrated against all *Candida* species, with higher MICs than micafungin, and like the other echinocandins is apparently fungistatic against *Aspergillus in vitro*. However *in vivo* it is fungicidal in *Candida* models[1] and also at high doses fungicidal in *Aspergillus* models.[2] Its extended spectrum is not yet documented. Single dose Phase 1 studies of intravenous compound

are completed and showed that the drug was well tolerated.[14] Phase 2 studies are planned.

In this study, we have confirmed that *in vivo C tropicalis* FA1572 is resistant to fluconazole but susceptible to amphotericin. We have further demonstrated that aminocandin at doses of ≥1.0 mg/kg has potent *in vivo* activity against this strain both in terms of improved survival and reduction in organ burden in a murine model.

It has previously been demonstrated that aminocandin is effective against systemic disease caused by multiple strains of *C. albicans* and that treatment was effective when drug levels in serum were more than four times the MIC (with maximal killing at 10 times the MIC).[1] In this study, doses of aminocandin in excess of 1.0 mg/kg/day were effective and it is likely that the peak serum drug levels exceeded 10 times the MIC during therapy (pharmacology was not performed in this study).[1] This would not have been the case with the lower doses of aminocandin (≤0.25 mg/kg/day) in which the peak serum concentration would not have exceeded four times the MIC; therefore these treatment groups as expected neither improved survival nor reduced organ burdens compared with control regimens.

The data presented here again demonstrate the efficacy of aminocandin in the treatment of invasive murine candidiasis and warrant further investigation of this agent.

Acknowledgements

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